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## **Table of Contents**

Front Cover.....	Preface
Standard Form 298.....	Preface
Foreword.....	Preface
Introduction.....	Page 1
Methods	
1) Subjects.....	Page 2
2) Study Design.....	Page 2
3) Statistical Analysis.....	Page 3
Results.....	Page 3
Conclusions.....	Page 3
Bibliography.....	Page 5
Appendices	
1) Appendix A- Figure Legend.....	Page 8
2) Appendix B- Table 1.....	Page 9
3) Appendix C- Figure. 1.....	Page 10

## **Introduction**

With increasing numbers of female members of the US Armed Forces, aspects of physical fitness unique to women are increasingly important to military readiness and thus to the success of the overall mission. In addition to the physiologic and psychologic stressors common to all military personnel, female members are also subject to hormonal fluctuations during the course of the female menstrual cycle. There is a growing body of knowledge regarding the effects of estrogens and progestins on a large number of metabolic processes, including carbohydrate (1-4) and lipid metabolism (5), thermoregulation(6), maintenance of extracellular plasma volume(7), ventilation (8), muscle fiber strength (9), maximal aerobic capacity and generalized endurance (10-19). Cyclical changes in strength, generalized endurance and maximal aerobic capacity may be of extreme importance in combat and/or other situations of extreme stress.

There have been a number of studies investigating the effects of the menstrual cycle on various measures of exercise performance (10-19). The results of these studies have been inconsistent, perhaps due to a number of methodological problems. Many early trials failed to document ovulation with hormone levels. Virtually every study has included fewer than 15 subjects and measured numerous outcome variables leading to problems with both Type I and Type II error. Most investigators have studied women during the mid-follicular phase when progesterone and estradiol levels are low and during the mid-luteal phase when progesterone and estradiol levels are elevated, but not at mid-cycle during ovulation when estradiol levels rise while progesterone levels remain low. Since progesterone has many "anti-estrogenic" properties, important physiologic effects of these two hormones may be masked by studying only the mid-follicular and mid-luteal time periods (12).

Recent studies have demonstrated important effects of estrogens on human vascular reactivity (20) which may be important to exercise performance. At times of peak exertion, the peripheral arterioles (resistance vessels) dilate maximally in order to increase overall perfusion by the process of autoregulation. The larger muscular arteries dilate in response to the increased blood flow, a process termed, "flow-mediated vasodilation." Flow-mediated vasodilation is accomplished by the release of a locally acting vasodilator hormone—endothelium-derived relaxing factor or EDRF-- from healthy vascular endothelial cells and this process is termed "endothelium-dependent vasodilation"(21). In the human coronary arteries and peripheral circulation, endothelium-dependent vasodilation is blunted in subjects with established atherosclerosis as well as in those with risk factors for atherosclerosis such as hypercholesterolemia (21-33). In healthy post-menopausal females, the acute administration of intravenous conjugated estrogen leads to enhancement of endothelium-dependent vasodilation (20). It is not known whether the endogenous cycling of estradiol and progesterone produces cyclical changes in endothelium-dependent vasodilation. Furthermore, it is not known whether changes in endothelium-dependent vasodilation correlate with changes in exercise performance. Finally, it is not known whether hypercholesterolemia modulates the cyclical hormonal effects due to blunting of baseline endothelium-dependent vasodilation.

We hypothesize that cyclical changes in estradiol and progesterone during the human female menstrual cycle are associated with changes in endothelium-dependent vasodilation, with the most pronounced vasodilation occurring during the mid-cycle when estradiol is unopposed by progesterone. We also believe that cyclical changes in endothelium-dependent vasodilation will correlate with cyclical changes in exercise performance and serum cholesterol levels.

## **Methods**

### **Subjects**

We recruited 28 eumenorrheic, females on active military duty at Wilford Hall Medical Center, San Antonio, TX. Eumenorrhea was defined as duration of menstrual flow 4 +/- 2 days, cycling at 28 +/- 2 days over the previous 6 months. Eight women originally enrolled in the study were later excluded secondary to not completing all three phases of the study (n=3), incomplete laboratory data (n=1) or inappropriate fluctuations in serum hormone levels (n=4) defined as lack of an estrogen surge at mid-cycle or progesterone surge during the luteal phase. Hence, the final study group consisted of 20 subjects. Exclusion criteria included pregnancy, current use of oral contraceptives, current cigarette use, and the presence of a medical condition that required regular medication. The non-pregnant state was confirmed by urine beta-HCG. All subjects provided written informed consent.

### **Study Design**

Blood sampling, determination of maximum oxygen consumption, endurance time on an anaerobic speed test (AST) and measurement of flow-mediated vasodilation and nitroglycerin-induced dilation were performed at three times during the menstrual cycle: the follicular phase (days 1-3), ovulation (days 11-13) and the luteal phase (days 23-25), with the onset of menses defined as day 0. Serum estradiol and progesterone levels were measured by sensitive radioimmunoassay (estradiol: Tosoh AIA-1200; progesterone: Isodata-520). Lipid profiles were measured using a standard lipid assay (Boehringer-Manheim Hitachi 917).

Flow mediated vasodilation and nitroglycerin-induced dilation were performed using high-resolution ultrasonography. Details of the technique used in our laboratory are published elsewhere (34). Briefly, images were obtained using a Hewlett-Packard 7.5 MHz linear array transducer and a sonos 2500 cardiac ultrasound system with vascular imaging software (Hewlett-Packard, Andover, Massachusetts). After subjects rested quietly for at least 5 minutes, baseline 2-dimensional longitudinal images of the left brachial artery were obtained at a location between 2 and 10 cm above the antecubital fossa. Next a blood pressure cuff placed proximal to the imaging transducer was inflated to suprasystolic pressure for 5 minutes. After rapid deflation of the cuff, flow-mediated vasodilation was assessed by imaging the brachial artery continuously with the transducer maintained at its original location during reactive hyperemia. Subjects rested quietly for 5 minutes, and endothelium-independent vasodilation was assessed after administration of sublingual nitroglycerin spray (0.4 mg). In addition to 2-dimensional imaging, flow

was estimated by continuous wave doppler for each of the scans. All images were recorded on super VHS tape for later analysis. For each subject, a technician blinded to order of the scans identified comparable segments for analysis using anatomic landmarks such as a vessel branchpoint. Three diameter measurements were obtained during each of 3 consecutive cardiac cycles at end-diastole. For the scans during reactive hyperemia, images were selected 60 seconds after release of the blood pressure cuff. The 9 measurements for each scan were averaged for subsequent statistical comparison.

Maximum oxygen consumption was determined using a stationary bicycle at a continuous speed, ramped resistance protocol and an on-line gas analysis system (Sensormetrics). The anaerobic speed test was determined by having subjects run at 8 mph at a 20% grade on a treadmill until fatigue, with the overall time measured in seconds (35). Subjects rested at least 1.5 hours following maximum oxygen consumption determination, prior to the AST determination.

### **Statistical Analysis**

Data obtained during the three phases of the menstrual cycle were analyzed with multivariate repeated-measures ANOVA. Linear regression was used to correlate flow-mediated vasodilation with serum estradiol, progesterone, lipid levels, maximum oxygen consumption and AST. Data are expressed as mean +/- SEM, and a p < 0.05 was considered statistically significant. Analyses were performed using Systat 5.2 for Macintosh.

### **Results**

The mean age of the subjects was 29.3 years (range 21-38). Compared to baseline, doppler estimated flow was increased during reactive hyperemia and after sublingual nitroglycerin for all subjects. Table 1 displays the results of hormone levels, lipid levels, and flow-mediated vasodilation and nitroglycerin-induced dilation. Figure 1 displays results of flow-mediated vasodilation and nitroglycerin-induced dilation for each individual patient. Flow-mediated vasodilation was highest during mid-cycle when estrogen was unopposed and was similar during the follicular and luteal phase ( $p = 0.041$ ). There was a similar trend noted for nitroglycerin-induced dilation, with the most pronounced vasodilation in mid-cycle compared with the follicular phase or the luteal phase ( $p = 0.059$ ). The only significant correlate of flow-mediated vasodilation was the serum progesterone level ( $R = -0.261$ ,  $p = 0.27$ ). Lipid levels, maximum oxygen consumption and AST times did not change significantly during the three phases of the menstrual cycle.

### **Conclusions**

Endothelial function estimated by flow mediated vasodilation of the brachial artery is 36% greater during the estrogen surge at mid-cycle than during the follicular phase when estrogen and progesterone levels are low. When both estrogen and progesterone levels are elevated during the luteal phase, flow-mediated vasodilation is similar to when neither hormone level is elevated during early follicular phase. In

addition, we found a significant negative correlation between progesterone levels and flow mediated vasodilation. Our data suggest a similar cyclical pattern for nitroglycerin-induced vasodilation, a measure of endothelium-independent vasodilation, although the p value was of borderline significance. There was no significant difference in serum lipid levels during the menstrual cycle to explain our findings. Exercise performance also did not appear to differ significantly during the menstrual cycle. These results indicate that cyclic changes in estrogen and progesterone have no effect on exercise performance. However, the lack of variation in performance may reflect the low number of patients which were included in the study or the way in which we assessed physical endurance.

The enhanced release of nitric oxide from endothelial cells is the primary mechanism by which the brachial artery dilates during reactive hyperemia (36). There is good evidence that ovarian hormones modulate endothelial function. In cultured endothelial cells, 17 beta-estradiol enhance the expression of constitutive nitric oxide synthase (37). In rabbits, ovarian hormones have been shown to regulate the unstimulated release of nitric oxide (38). In a dog model, Miller and Vanhoutte showed that progesterone antagonizes the enhanced coronary artery endothelium-dependent vasodilation associated with estrogen administration (39). In observational human study comparing endothelium-dependent vasodilation in postmenopausal women receiving hormone replacement therapy with estrogen alone or combination progesterone and estrogen, the addition of progesterone was associated with 18% lower flow-mediated vasodilation of the brachial artery, although this difference was not significant perhaps due to a small sample size (40). In contrast to the results of this study, Hashimoto and colleagues found that flow-mediated vasodilation is enhanced in human subjects whenever estradiol levels are elevated, regardless of the level of serum progesterone (41).

In addition to effects modulated by the endothelium, estrogen also potentiates endothelium-independent vasorelaxation. For example, Chester and colleagues demonstrated that 17 beta-estradiol directly induces relaxation in atherosclerosis-free human coronary arteries harvested from patients undergoing heart or heart-lung transplantation (42). Another group showed that exogenous estrogen potentiates endothelium-independent vasodilation in subjects with risk factors for vascular dysfunction (43). Hashimoto demonstrated that endothelium-independent vasodilation was augmented in phases of the menstrual cycle associated with elevated estradiol levels. However, in contrast to the results of this study, progesterone did not attenuate this beneficial effect (41).

In summary, our study confirms the findings of previous investigators that endogenous estradiol has a beneficial effect on endothelium-dependent and independent vasorelaxation. However, our data suggest that progesterone antagonizes the beneficial effect of estrogen on vascular reactivity, and this observation may be important in the development of hormone replacement strategies that minimize cardiovascular risk in post-menopausal women.

## **Bibliography**

1. Ahmed-Sorour H, Bailey CJ. Role of ovarian hormones in the long-term control of glucose homeostasis, glycogen formation and gluconeogenesis. *Ann Nutr Metab* 25:208-212, 1981.
2. Kendrick ZV, Steffen CA, Rumsey WL, and Goldberg DI. Effect of estradiol on tissue glycogen metabolism in exercised oophorectomized rats. *J Appl Physiol* 63:492-496, 1987.
3. Matute ML and Kalkhoff RL. Sex steroid influence on hepatic gluconeogenesis and glycogen formation. *Endocrinology* 92:762-768, 1973.
4. Nicklas BJ, Hackney AC, and Sharp RL. The menstrual cycle and exercise: performance, muscle glycogen and substrate responses. *Int J Sports Med* 10:264-269, 1989.
5. Bunt JC. Metabolic actions of estradiol: significance for acute and chronic exercise response. *Med. Sci. Sports Exerc* 22:286-290, 1990.
6. Hessemer V and Bruck K. Influence of menstrual cycle on shivering, skin blood flow and sweating responses measured at night. *J Appl Physiol* 59:1902-1910, 1985.
7. Fortney SM, Beckett WS, Carpenter AJ, et al. Changes in plasma volume during bed rest: effects of the menstrual cycle and estrogen administration. *J Appl Physiol* 65:525-533, 1988.
8. Schoene RB, Robertson HT, Pierson DJ, and Peterson AP. Respiratory drives and exercise in menstrual cycles of athletic and nonathletic women. *J Appl Physiol* 50:1300-1305, 1981.
9. Dibrezzo RI, Fort L, and Brown B. Relationships among strength, endurance, weight and body fat during three phases of the menstrual cycle. *J Sports Med Phys Fitness* 31:89-94, 1991.
10. Lebrun CM, McKenzie DC, Prio Cr, and Taunton JE. Effects of menstrual cycle phase on athletic performance. *Sci Sports Exerc* 1995;27:437-444.
11. Lebrun CM. Effect of the Different Phases of the Menstrual Cycle and Oral Contraceptives on Athletic Performance. *Sports Medicine* 1993;16:400-430.
12. Lebrun CM. The effect of the Phase of the Menstrual Cycle and The Birth Control Pill on Athletic Performance. *Clinics In Sports Medicine* 1994;13:419-41.
13. Eston RG. The Regular Menstrual Cycle and Athletic Performance. *Sports Medicine* 1984;1:431-445.
14. De Souza MJ, Maguire MS, Rubin KR, Maresh CM. Effects of menstrual phase and amenorrhea on exercise performance in runners. *Med Sci Sports Exerc* 1990;22:575-580.
15. Bonen A., Haynes FJ, Watson-Wright W, et al. Effects of menstrual cycle on metabolic responses to exercise. *J Appl Physiol* 55:1506-1513, 1983.
16. Dombrovsky ML, Bonekat HW, Williams TJ, and Staats BJ. Exercise performance and ventilatory response in the menstrual cycle. *Med Sci Sports Exerc* 19:111-117, 1987.
17. Hackney AC., Curley CS, and Nicklas BJ. Physiologic responses to submaximal exercise at the mid-follicular, ovulatory, and mid-luteal phases of the menstrual cycle. *Scand. J Med Sci Sport* 1:94-98, 1991.

18. Jurkowski JEH, Jones NL, Toews CJ, and Sutton JR. Effects of menstrual cycle on blood lactate, O<sub>2</sub> delivery and performance during exercise. *J Appl Physiol* 51:1493-1499, 1981.
19. Nicklas BJ, Hackney AC, and Sharp RL. The menstrual cycle and exercise: performance, muscle glycogen and substrate responses. *Int J Sports Med* 10:264-269, 1989.
20. Gerhard MD, Roddy M, Knab ST, Creager SJ, Creager MA. Acute Estrogen Administration Improves Endothelium-Dependent Vasodilation in Postmenopausal Women: *Circulation* 1994;90(Suppl I):I-86.
21. Nabel E, Selwyn A, Ganz P. Mechanisms of flow-mediated vasodilation in human epicardial coronary arteries: Impairment by atherosclerosis. *J Am Coll Cardiol* 1990;16:349.
22. Lieberman E, Uehata A, Polak J, et al. Flow-mediated vasodilation is impaired in the brachial artery in patients with coronary artery disease or with diabetes mellitus. *Clin Res* 1993;41:217.
23. Nabel E, Ganz P, Gordon J, Alexander R, Selwyn A: Dilation of normal and constriction of atherosclerotic coronary arteries caused by the cold pressor test. *Circulation* 1988;77:43-52.
24. Yeung A, Vekshtein V, Krantz D, Vita J, Ryan TJ, Ganz P, Selwyn A: The effect of atherosclerosis on the vasomotor response to coronary arteries to mental stress. *N Engl J Med* 1991 In press.
25. Gordon J, Ganz P, Nabel E, Zebede J, Mudge G, Alexander R, Selwyn A: Atherosclerosis and endothelial function influence the coronary vasomotor response to exercise. *J Clin Invest* 1989;83:1946-1952.
26. Nabel E, Selwyn A, Ganz P: Mechanisms of flow-mediated vasodilation in human epicardial coronary arteries: Impairment by atherosclerosis. *J Am Coll Cardiol* 1990;16:349-356.
27. Ludmer P, Selwyn A, Shook T, Wayne R, Mudge GJ, Alexander R, Ganz P: Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. *N Engl J Med* 1986;315:1046.
28. Vita J, Treasure C, Nabel E, McLenahan J, Fish R, Yeung A, Vekshtein V, Selwyn A, Ganz P: The coronary vasomotor response to acetylcholine relates to risk factors for coronary artery disease. *Circulation* 1990;81:491-497.
29. Liao J, Bettman M, Sandor T, Tucker J, Coleman S, Creager M: Differential impairment of vasodilator responsiveness of peripheral resistance and conduit vessels in humans with atherosclerosis. *Circ Res* 1991;68:1027-1034.
30. Creager M, Cooke J, Mendelsohn M, Gallagher S, Coleman S, Loscalzo J, Dzau V: Impaired vasodilation of forearm resistance vessels in hypercholesterolemic humans. *j Clin Invest* 1990;86:228-234.
31. Celermajer D, Sorensen K, Gooch V, et al. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet* 1992;340:1111.
32. Celermajer D, Sorensen K, Georgakopoulos D, et al. Cigarette smoking is associated with dose-related and potentially reversible impairment of endothelium-dependent dilation in healthy young adults. *Circulation* 1993;88:2149.

33. Nabel E, Selwyn A, Ganz P. Large coronary arteries in humans are responsive to changing blood flow: an endothelium-dependent mechanism that fails in patients with atherosclerosis. *J Am Coll Cardiol* 1990;16:349.
34. Andrews T, Whitney E, Green G, Kalenian R, Personius B. Effect of gemfibrozil +/- niacin +/- cholestyramine on endothelial function in patients with serum low-density lipoprotein cholesterol levels <160mg/dl and high density lipoprotein cholesterol levels <40 mg/dl. *Am J Cardiol* 1997; 80:831-5.
35. Cunningham, D. A. And J. A. Faulkner. The effect of training on aerobic and anaerobic metabolism during a short exhaustive run. *Med Sci. Sports Exerc.* 1:65-69, 1969.
36. Joannides R, Haefeli W, Linder L, Richard V, Bakkali, E, Thuillez C, Luscher T. Nitric oxide is responsible for flow-dependent dilatation of human peripheral conduit arteries in vivo. *Circulation* 1995;91:1314-1319.
37. Hayashi T, Yamada K, Esaki T, Kuzuya M, Satake S, Ishikawa T, Hidaka H, Iguchi A. Estrogen increases endothelial nitric oxide by a receptor-mediated system. *Biochem Biophys Res Commun* 1995; 214:847-55.
38. Hayashi T, Fukuto J, Ignarro L, Chaudhuri G. Basal release of nitric oxide from aortic rings is greater in female rabbits than in male rabbits: implications for atherosclerosis. *Proc Natl Acad Sci USA* 1992;89:11259-63.
39. Miller V, Vanhoutte P. Progesterone and modulation of endothelium-dependent response in canine coronary arteries. *Am J Physiol* 1991;261:R1022-R1027.
40. McCrohon J, Adams M, McCredie R, Robinson J, Pike A, Abbey M, Keech A, Celermajer D. Hormone replacement therapy is associated with improved arterial physiology in healthy post-menopausal women. *Clin Endocrinol* 1996;45:435-41.
41. Hashimoto M, Akishita M, Eto M, Ishikawa M, Kozaki K, Toba K, Sagara Y, Taketani Y, Orimo H, Ouchi Y. Modulation of endothelium-dependent flow-mediated dilatation of brachial artery by sex and menstrual cycle. *Circulation* 1995; 92:3431-5.
42. Chester A, Jiang C, Borland J, Yacoub M, Collines P. Oestrogen relaxes human epicardial coronary arteries through non-endothelium-dependent mechanisms. *Coron Artery Dis* 1995;6:417-22.

## **Appendix A**

### **FIGURE LEGEND**

Values plotted for flow-mediated vasodilation for each subject during three phases of the menstrual cycle.

## Appendix B

**TABLE 1: Flow-mediated vasodilation, nitroglycerin-induced vasodilation, hormone and lipid levels during three phases of the menstrual cycle**

	<u>Follicular</u>	<u>Mid-cycle</u>	<u>Luteal</u>	<u>p value</u>
FMD (%)	8.0±1.1	10.9±1.4	7.6±1.1	0.041
TNG (%)	24.0±1.7	28.2±1.8	23.6±1.6	0.059
Estradiol (pmol/L)	49±4.6	174±26.6	157±14.1	<0.001
Progesterone (nmol/L)	0.7±0.07	0.9±0.16	9.4±1.0	<0.001
LDL-chol (mg/dl)	94.2±5.3	96.3±5.3	91.3±5.4	NS
HDL-chol (mg/dl)	47.2±1.7	49.5±1.7	49.8±2.0	NS
Triglycerides (mg.dl)	67.3±5.8	77.8±9.6	71.6±6.7	NS

### **Abbreviations**

**FMD = flow-mediated vasodilation**

**HDL-chol=HDL cholesterol**

**LDL-chol = LDL-cholesterol**

**NS = not significant**

**TNG = nitroglycerin-induced vasodilation,**

## Appendix C

